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VARIATIONS AND TAXONOMIC SIGNIFICANCE OF THE CHROMOSOME NUMBERS IN THE NEARCTIC SPECIES OF THE GENUS *LEPTOTHORAX* (S.S.) (FORMICIDAE: HYMENOPTERA)

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SUMMARY — The taxonomic situation of the genus *Leptothorax* (sensu stricto = subgenus *Leptothorax* sensu Smith 1950) appears particularly puzzling in North America when using morphological characters alone. This paper analyses the taxonomic impact of the complex variations observed in the chromosome numbers of the presently recognized species. The method of Imai modified for some details was applied to specimens obtained from colonies reared in laboratory. These were collected between 1978 and 1984, in different regions of Québec and in Alberta for Canada, in New Hampshire and Utah for USA. Over 250 slides of testes (male pupae) or brain (female prepupae) were prepared. Results for the nearctic species are compared to European data. For the traditional *muscorum* taxon as applied in North America the haploid numbers varied from 15 to 23 including supernumerary chromosomes. At least four distinct species can be extracted from these results. Male diploidy is documented. Different tendencies are stressed between the actual subgenera *Leptothorax* and *Myrafant*.

INTRODUCTION

In our revision of the northern genera of the tribe Leptothoracini (see Francoeur *et al.* 1985), we have undertaken the study of the ant genus *Leptothorax* (sensu stricto = subgenus *Leptothorax* sensu Smith 1950). The taxonomic situation of this genus appears particularly puzzling in North America when using morphological characters alone. The karyotypes of the constituent species (old and new) were examined in the hope to untangle this situation. Special attention was paid to *L. muscorum* which shows some more or less typical phenototypes in different regions of its large geographical distribution. Do the observed phenotypes correspond to variations in chromosome numbers? If so, is it possible to match different chromosome numbers to

precise morphological caracteristics? This paper analyses the taxonomic impact of the variations occuring in presently recognized species.

Up to recently the data available on chromosome numbers in the genus *Leptothorax* (s.s.) were rare and pertaining only to *L. acervorum* which has $n = 13$ (CROZIER 1975; HAUSCHTECK-JUNGEN and JUNGEN 1983). FISCHER in 1987 added a whole set of new data. They are compared and evaluated with our results gained from different series in order to provide a basis for morphological interpretation and to establish taxonomic decisions.

MATERIALS AND METHODS

Colonies were kept in growth chambers with controlled climate. More than 75 colonies produced the male pupae or female prepupae used for the chromosome enumeration. From North America were available different forms traditionally allied to *Leptothorax muscorum*, and also the species *acervorum, crassipilis, sphagnicolus* and *retractus* (see FRANCOEUR 1986 for the description of the last two species). These colonies were collected between 1978 and 1984, in different region of Québec and in Alberta for Canada, in New Hampshire and Utah for USA (Table 1). European colonies of *L. acervorum, gredleri* and *muscorum* were reared as well for comparason.

Enumeration of chromosomes was made on more than 250 slides of testes (male pupae) or brain (female prepupae) preparations. In all cases the Imai technique (IMAI *et al.* 1977) was applied. One slide for each specimen, pupa or prepupa, were prepared. The second step of this technique was modified as follows: once removed the testes were transfered directly in 2 or 3 drops of the hypotonic solution already applied on a microscope slide (instead of a second depression slide). This minor modification eliminates an often risky transfer of the organs from a depression slide to another. Step six produces a better fixation by adding 5 drops of freshly-prepared fixative II instead of only 2 as indicated.

RESULTS AND DISCUSSION

Results are examined in two folds: first those obtained with the *muscorum* complex and second those dealing with the other species of *Leptothorax.*

The «muscorum» complex.

Table 1 shows the chromosome numbers of 161 males (+1 female prepupa) produced by 63 colonies identified as *L. «muscorum»*. Individuals from these colonies showed various phenotypes handily named: «small brown», «big black», «small yellow», etc. The observed haploid numbers in this survey were 16 (Fig. 1a). 17, 18, 22 and 23. Number of slides used for enumeration and number of slides with less than 10 metaphases corresponding to the

TABLE 1 - *Chromosome numbers in males of the «muscorum» complex.*

Coll. no.	Locality	Region	Date	*n* (2*n*)	Slides nb.	< 10[1]	Species
CAF 11106	Mont Rigaud	Qué., CDN	83 07 08	16, 17	1,1		*muscorum*
CAF 11123	Saint-Siméon	Qué., CDN	83 06 27	16, 17	1		*muscorum*
CAF 11142	Tadoussac	Qué., CDN	83 06 25	16, 17	2, 1		*muscorum*
CAB 8969a	Maligne Canyon	Alb., CDN	79 08 16	17	1	1	*muscorum*
CAB 08769	MT. Edith Cavill Road	Alb., CDN	79 08 19	17 (34)	5		*muscorum*
CAF 09871	Grandes-Bergeronnes	Qué., CDN	81 08 10	17	2	1	*muscorum*
CAF 10240	Brighton	Utah, USA	82 08 16	17	5		*muscorum*
CAB 10826	Jordan River	Utah, USA	82 08 16	17 (34)	3		*muscorum*
CAF 10289	Tony Gr. Lake, L.C.	Utah, USA	82 08 20	17	2	1	*muscorum*
CAF 10385	Sink Hollow, L.C.	Utah, USA	82 08 20	17	3	1	*muscorum*
CAB 10859	Logan Canyon	Utah, USA	82 08 20	17	4		*muscorum*
CAB 10875	Mt. Evans Road, Denver	Col., USA	82 08 22	17	2	2	*muscorum*
CAF 11037	Magpie, Côte Nord	Qué., CDN	83 06 09	17	1		*muscorum*
CAF 11269	Baie Comeau	Qué., CDN	83 06 10	17			*muscorum*
CAF 11190	Mont Washington	N.H., USA	84 06 12	17	3		*muscorum*
CAF 11192	Mont Washington	N.H., USA	84 06 12	17	5	4	*muscorum*
CAF 11101	Parc des Grands Jardins	Qué., CDN	83 07 01	17, 18	2, 2	2	*muscorum*
CAF 11188	Mont Washington	N.H., USA	84 06 12	17, 18	1, 2	1	*muscorum*
CAF 11193	Mont Washington	N.H., USA	84 06 12	17, 18	2, 2		*muscorum*
CAF 11195	Mont Washington	N.H., USA	84 06 12	17, 18	2, 4	1	*muscorum*
CAF 11196	Mont Washington	N.H., USA	84 06 12	17, 18	1, 1	1	*muscorum*
CAF 09568	Poste-de-la-Baleine, T.N.Q.	Qué., CDN	80 07 13	17, 18 (34)	2	1	*muscorum*
CAB 08202	Farmborough	Qué., CDN	78 06 06	18	2	2	*muscorum*
CAB 08709	Maligne Canyon	Alb., CDN	79 08 17	18	3		*muscorum*
CAB 08710	Maligne Canyon	Alb., CDN	79 08 17	18	3		*muscorum*
CAF 09525	Saint-Ambroise	Qué., CDN	80 06 25	18	1		*muscorum*
CAF 09560	Fort-Chimo, T.N.Q.	Qué., CDN	80 07 08	18	1		*muscorum*
CAF 09896	Grande Rivière, T.N.Q.	Qué., CDN	81 06 27	18	4		*muscorum*
CAF 10246	Brighton	Utah, USA	82 08 16	18	1		*muscorum*
CAB 10849	Logan Canyon	Utah, USA	82 08 19	18	3		*muscorum*
CAF 10278	Sink Hollow, L.C.	Utah, USA	82 08 20	18	2	1	*muscorum*
CAB 10857	Logan Canyon	Utah, USA	82 08 20	18	4		*muscorum*
CAB 10874	Mt. Evans Road, Denver	Col., USA	82 08 22	18	1	1	*muscorum*
CAB 10873	Mt. Evans Road, Denver	Col., USA	82 08 22	18	3		*muscorum*
CAF 10984	Frau Nauses, Hessia	RFA	83 05 27	18	1		*muscorum*
CAF 10977	Saint-Augustin	Qué., CDN	83 05 31	18	2		*muscorum*

(continued)

TABLE 1 *(continued)*

Coll. no.	Locality	Region	Date	n ($2n$)	Slides nb.	< 10[1]	Species
CAF 11268	La Baie	Qué., CDN	83 06 05	18			*muscorum*
CAF 11021	Coulée à Dominique, C.N.	Qué., CDN	83 06 07	18	3	1	*muscorum*
CAF 11009	Pte-aux-Anglais, C.N.	Qué., CDN	83 06 10	18	4	2	*muscorum*
CAF 11086	Parc des Grands Jardins	Qué., CDN	83 07 01	18	3	2	*muscorum*
CAF 11087	Parc des Grands Jardins	Qué., CDN	83 07 01	18	2	1	*muscorum*
CAF 11095	Parc des Grands Jardins	Qué., CDN	83 07 01	18	1		*muscorum*
CAF 11166	Mont Washington	N.H., USA	84 06 11	18	1	1	*muscorum*
CAF 11189	Mont Washington	N.H., USA	84 06 12	18	2		*muscorum*
CAF 11854	Miguasha, Gaspésie	Qué., CDN	85 07 16	18	2	1	*muscorum*
CAF 11856	Miguasha, Gaspésie	Qué., CDN	85 07 16	18	2		*muscorum*
CAF 11859	Miguasha, Gaspésie	Qué., CDN	85 07 16	18	1		*muscorum*
CAF 11860	Miguasha, Gaspésie	Qué., CDN	85 07 16	18	1		*muscorum*
CAF 11861	Miguasha, Gaspésie	Qué., CDN	85 07 16	18	4		*muscorum*
CAF 09493	Chicoutini	Qué., CDN	80 05 23	(36)	(2)	1	*muscorum*
CAB 08666	Maligne Canyon	Alb., CDN	79 08 16	(36)	1	1	*muscorum*
CAF 10290	Tony Gr. Lake, L.C.	Utah, USA	82 08 20	(44)	(2)		*muscorum*
CAF 10288	Tony Gr. Lake, L.C.	Utah, USA	82 08 20	(36 –)	(3)	3	*muscorum*
CAF 10261	Right Fork, L.C.	Utah, USA	82 08 19	18 (35 –)	1 (1)	2	*muscorum*
CAF 09895	Grande Rivière, T.N.Q.	Qué., CDN	81 06 27	18 (36)	1 (2)		*muscorum*
CAB 8665	Maligne Canyon	Alb., CDN	79 08 16	18 (36)	4		*muscorum*
CAF 11183	Lac Mégantic	Qué., CDN	84 06 14	18 (36 +)	1 (1)		*muscorum*
CAF 10470	Lac à l'Eau Claire, T.N.Q.	Qué., CDN	82 08 05	17, 18, 23	1, 2, 2		*muscorum*
CAF 11094	Parc des Grands Jardins	Qué., CDN	83 07 01	23 (44 –)	3 (1)		*muscorum*
CAF 10234	Jordan River	Utah, USA	82 08 16	17	2	1	spec. 1
CAF 10244	Jordan River	Utah, USA	82 08 16	17	4	2	spec. 1
CAF 11001	Mingan, C.N.	Qué., CDN	83 06 08	15 (30 –)	1 (2)	3	spec. A
CAF 11100	Parc des Grands Jardins	Qué., CDN	83 07 01	15	1		spec. A
CAF 11140	Tadoussac	Qué., CDN	83 06 25	15	1		spec. A
CAF 11270	Tadoussac	Qué., CDN	83 06 25	15			spec. A
CAF 11135	Saint-Siméon	Qué., CDN	83 06 27	16	1		spec. A
CAF 11139	Tadoussac	Qué., CDN	83 06 25	16	3		spec. A

[1]10: number of slides with less than 10 metaphases with the right n or $2n$.
C.N. = Côte Nord, Québec, Canada.
NQ. = Territoire-du-Nouveau-Québec, Canada.
L.C.: Logan Canyon, Utah, United States.

haploid and/or the diploid number are indicated. In fact 120 of the 161 slides (74.5%) have more than 10 determining metaphases; sometimes this number reached 50.

European-like form. — The Buschinger's team was the first to study the chromosomes of *muscorum* populations; the colonies were collected in Federal Republic of Germany (FISCHER 1987). The haploid number found in males of these colonies was $n = 17$. From the 63 colonies sampled in North America only 15 produced males with 17 chromosomes. The two bigger chromosomes in Fig. 1b are similar to those of the European *muscorum* illustrated in the Fig. 3f in the FISCHER thesis (1987). Individuals of these colonies mostly correspond to the «small brown» phenotype.

However, one German colony (10984, Table 1) bred in the Laboratoire de biosystématique had $n = 18$.

«Big black» form. — Males of 31 colonies sampled in North America had $n = 18$ (Fig. 1c). In these colonies ergatomorphs and gynomorphs are usually taller and darker than in the European form. Until now the «big black» seems to be restricted to North America. It is the the only form found north of the 51 st parallel; southward it coexists with the Europeanlike form.

«Intermorphic queen» form. — The intermorphic queen form (= *Leptothorax* sp. A) has already been studied by HEINZE and BUSCHINGER (1987, 1988*a*, 1988*b*, 1989). At the Laboratoire de biosystématique, six colonies sampled in Québec, mainly near Tadoussac and Saint-Siméon (Charlevoix-Est and Saguenay co.), produced males with haploid number 15 (Fig. 2a) or 16. An intermorph was assuming the function of queen in three of these colonies. Such an important role of the intermorph is rather unusual in the genus *Leptothorax*. This, together with a different chromosome number and some distinctive morphological characters support the conclusion that this form is in fact a new species (to be describe elsewhere). The phenomenon of queen polymorphism expressed in this species has been analysed by HEINZE and BUSCHINGER (1987). The quantitative importance of these ergatogynes was examined in different colonies of this new species and compared with similar data on other species of *Leptothorax* and *Formicoxenus* (HEINZE and BUSCHINGER 1989).

16 or 17-chromosome form. — One colony from Tadoussac (CAF 11142) produced 3 males with 16 or 17 chromosomes: $n = 16$ in 17 of the 19 observed metaphases (89.5%) and in 12/21 (57.1%); $n = 17$ in 46/58 (79.3%). The same divergence is shown by two males taken from a colonie sampled at mont Rigaud (CAF 11106): $n = 16$ in 30/40 (75%) and $n = 17$ in 34/36 (94.4%). These results are masking the issue, showing an intermediate position between the preceding new species ($n = 15$ or 16) and the different forms of *muscorum* ($n = 17$, 18, 22 and 23).

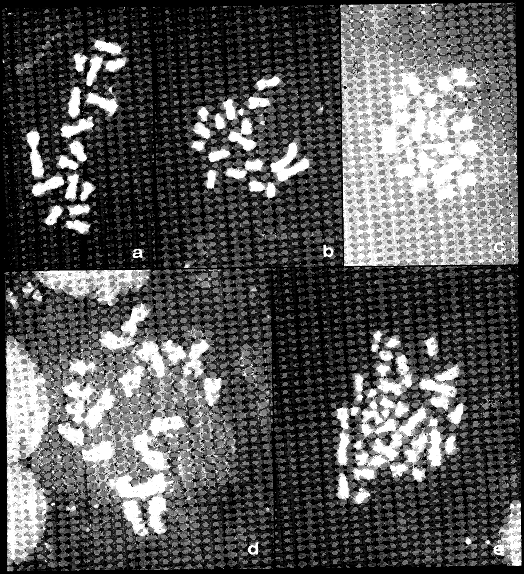

Fig. 1. — Metaphasic plates from different populations of the *Leptothorax muscorum* complex. (a) Saint-Siméon. Québec, (CAF 11123; $n=16$); (b) Mt Washington, N.H., (CAF 11190; $n=17$); (c) Côte Nord, Québec, (CAF 11021; $n=18$); (d) Parc des Grands Jardins, Québec, (CAF 11094; $n=23^+$); (e) Logan Canyon, Utah, (CAF 10290; $2n=44$). 1250×.

17 or 18-chromosome form. — In two samples from Québec (CAF 10470 and 11101) and four samples from New-Hampshire (CAF 11188, 11193, 11195 and 11196), we have found 17-chromosome males together with 18-chromosome males. The male pupae used for these tests were coming from larvae sampled in the field some months before except for the colony 10470 (with 6 gynomorphs) which was running its fourth artificially compressed season cycle. These larvae were kept in growth chamber and spent the winter in artificial conditions. It is then impossible to know how many queens were present in each colony at the sampling time. On the other hand it has been established by HEINZE and BUSCHINGER (1988*b*) that *Leptothorax* sp. A colonies are functionally monogyne; other phenotypes may be polygynous, including the «big black». We may here suppose the expression of a Robertsonian polymorphism; such variations of the chromosome number have already been observed in *Myrmecia forficata* (IMAI *et al.* 1977; $2n = 50$, 51), *Rhytidoponera metallica* (CROZIER 1969; $n = 22$ à 17), *R. maniae* (IMAI *et al.* 1977), *Pheidole nodus* (IMAI and KUBOTA 1975; $2n = 37$, 38, 39), *Aphaenogaster longiceps* (IMAI *et al.* 1977, $2n = 45$, 46) and possibly also in *Aphaenogaster rudis* (CROZIER 1977; $n = 20{,}22$).

Supernumerary chromosomes. — Eight males produced by colonies from Québec (CAF 10470 and 11094) and from Utah (CAF 10290) show a particular karyotype: 4 or 5 (rarely 6 or 7) supernumerary chromosomes are added to the standard 17 or 18 chromosomes (Fig. 1d). These B-chromosomes appear in all the metaphases where the enumeration is possible (20 metaphases in 10470, 69 in 11094). Moreover their number double in diploid males, showing their real nature. We then observe 8, 9 or 10 very small chromosomes added to 34, 35 or 36 regular chromosomes (37 metaphases in colony CAF 10290; Fig. 1e).

Among Insects supernumerary chromosomes are known since the beginning of the century; they have been observed mainly in Orthoptera and Coleoptera (JONES 1975). The discovery of the first B-chromosomes among Hymenoptera dates back to 1974 when IMAI found some mainly in the germ cells of males of *Leptothorax (Myrafant) spinosior.* Their number then varied between one to 12, the main frequencies being between 3 and 7. This number varied slightly from one cell to another for the same individual. Furthermore some individuals from the same colony had different modal numbers: for example 21 individuals from one colony show all the possibilities between zero and 8 B-chromosomes. FISCHER (1987) reports on B-chromosomes in *L.(M.) nigriceps* and in *L.(M.) curvispinosus,* probably between one and 14 in the latter species. Compared to these high variations, supernumerary chromosomes observed in the *L. muscorum* complex appear rather stable. Very small numbers of such B-chromosomes have also been observed in *Aphaenogaster rudis* (CROZIER 1975, 1977) and possibly in *Podomyrma adelaidae* (IMAI *et al.* 1977).

It seems that a relation exists between the 18-chromosome males and the 23-chromosome males.They were found together in the same colony (CAF

10470) from lac à L'Eau Claire (Territoire-du-Nouveau-Québec, lat.: 56°10'; long.: 74°25'). Moreover the two bigger submetacentric chromosomes of their karyotype are much alike. Besides, WHITE (1973) and JONES (1975) specified that usually B-chromosomes are only found in some individuals from a population: for example, an average between 30.7 and 37.2% in *Myrmeleotettix maculatus* (Orthoptera), of 64.5% in *Pseudococcus obscurus* (Hemiptera) and of 10.5% in *Trimerotropis sparsa* (Orthoptera). With *Leptothorax spinosior* IMAI (1974) observed supernumerary chromosomes in 116 of the 121 examined males, that is to say 96%. In the colony 10470 two out of five males had $n = 23$, two others had $n = 18$ and the fifth had $n = 17$. This colony possibly belongs to the first species that shows at the same time a Robertsonian polymorphism and B-chromosomes; according to IMAI *et al.* (1977) such a situation has never been observed.

A case similar to the «muscorum complex». — The problem faced with the «*muscorum*» complex appears similar to the one encountered by CROZIER (1977) in *Aphaenogaster rudis*. These two species are very common, show marked color variations and a large geographical distribution. In *A. rudis* the haploid number is 18, 20, 21 (with one supernumerary chromosome) or 22 (CROZIER 1977). A detailed study of the karyotypes and of some enzymes has demonstrated the presence of a species complex in *rudis*; we have here brought to the fore a problem which require the same approach with *muscorum*.

Electrophoresis of some enzymes has been made to get a better definition of the species hidden under the name «*muscorum*». The first results indicate that the nearctic forms are different from the European *muscorum* and would in fact represent at least two sympatric species, which show only slight differences in morphology and behaviour (HEINZE 1987).

Other species.

The known chromosome number of the *Leptothorax* (s.s.) and *Doronomyrmex* (a related genus) species are displayed in Table 2.

The presence of *L. acervorum* in the northern part of North America has been reported by FRANCOEUR (1983) for Québec and NIELSEN (1987) for Alaska. Near the tree limit in Québec 13 complete colonie have been sampled in localities far distant apart. The haploid number of chromosomes ($n = 13$) is identical to those usually observed in European populations and even the karyotypes (11 M + 2 A) are identical (see FISCHER 1987, Fig. 3, b and d, p. 35). Breeding tests between sexuals coming from Germany, Japan and Québec are in progress at Chicoutimi to check the gene flow between these different continental populations.

Recently described, *L. sphagnicolus* (FRANCOEUR 1986) have also $n = 13$ and the same karyotype as *L. acervorum* (see FISCHER 1987, Fig. 3, e, p. 35).

TABLE 2 - *Known numbers of chromosomes in the genera «Leptothorax» (s.s.) and «Doronomyrmex».*

Nearctic species	*n*	Palaeartic species	*n*
Leptothorax			
		gredleri	11 [3,4]
acervorum	13 [3,4]	*acervorum*	13 [2,3]
sphagnicolus	13 [3,4]		
faberi	15 [1,3]		
sp. A	15, 16 [4]		
sp. 1	17 [3,4]		
muscorum	17 [3,4]	*muscorum*	17 [3]
muscorum	18 [3,4]		
muscorum	17, 18 [4]		
muscorum	22, 23 [4]		
crassipilis	17, 18 [4]		
retractus	17, 18 [4]		
Doronomyrmex			
pocahontas	18 [3]		
		kutteri	23, 25 [3]
		pacis	26, 27 [3]
		goesswaldi	26, 28(?) [3]

[1] BUSCHINGER 1982.
[2] HAUSCHTECK-JUNGEN and JUNGEN 1983.
[3] FISCHER 1987.
[4] Laboratoire de biosystématique, UQAC, Chicoutimi.

With its typical habitus this species can be rather easily distinguished from any other species of the genus. Until now we have never found it outside the Saguenay-Lac-Saint-Jean region, in Québec. The name of the ant is derived from the fact that the colonies live in mosses (*Sphagnum* sp.) of spruce bog.

L. retractus has been described from specimens captured near Rouyn, Témiscamingue co., Québec. Collected near Saint-Siméon (Charlevoix-Est co., Québec), 5 males from the colony 11151 had $n = 17$ (Fig. 2b; 119/152 metaphases, 78.3%). On the other hand 4 males from the colony 10363 sampled on Beaver Mountain (Logan Canyon, Utah) had $n = 18$ (Fig. 2c; 77/108 metaphases, 71.3%). Individuals of the two colonies showed all the characters defining the *retractus* species. Nevertheless some differences were found in the genitalia between males of the two colonies (FRANCOEUR 1986).

The situation was quite the same with two colonies of *L. crassipilis* sampled in Western United States. The enumeration carried out on 5 males from Beaver Mountain (Logan Canyon, Utah; CAF 10357) showed an haploid number of $n = 18$ (Fig. 2e; 49/79 metaphases, 64.5) while 5 other males, from Ute Pass (Colorado; CAF 10191) had $n = 17$ (Fig. 2d; 45/63 metaphases, 71.4%).

Finally Table 2 summarizes the available knowledges on the chromosome

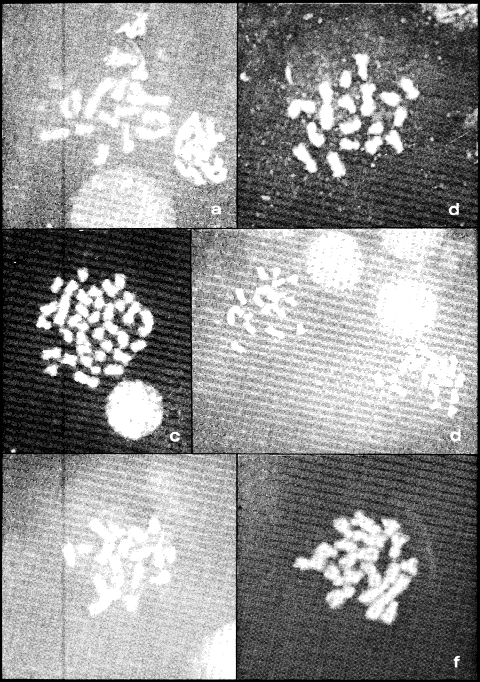

Fig. 2. — Metaphasic plates from different *Leptothorax* species. (a) *Leptothorax* spec. A. Parc des Grands Jardins, Québec (CAF 11100; $n = 15$); (b) *L. retractus*, Saint-Siméon, Québec, (CAF 11151; $n = 17$); (c) *L. retractus*, Logan Canyon, Utah, (CAF 10363; $2n = 36$); (d) *L. crassipilis*, Ute Pass, Colorado, (CAF 10191; $n = 17$); (e) *L. crassipilis*, Logan Canyon, Utah, (CAF 10357; $n = 18$); (f) *Leptothorax* spec. 1, Jordan River, Utah, (CAF 10244; $n = 17$).

numbers of the messy *muscorum* complex. Species a, with a haploid number of 15 or 16 will be described soon. Its particular biology is under investigation at the Institut für Zoologie of Darmstadt (HEINZE and BUSCHINGER 1988*a*, 1988*b*, 1989). Species 1, with a haploid number of 17, is a concolorous reddish brown ant. It is known only from the western part of North America. Concerning the other forms of the complex, it should be possible at least in part to combine the different karyotypes with precise phenotypes, when the study of the basic morphology is completed. Biogeographic and ecological data should also help to resolve the tangle.

«Leptothorax» (s.s.) versus «Myrafant».

The data now available show an apparently different tendency in the variation of the chromosome numbers in these two taxa. The haploid number of chromosomes in the genus *Leptothorax* (s.s.) (see FRANCOEUR *et al.* 1985 for taxonomic aspects) varies from 11 in the European species *gredleri* to 23 in two populations of the *muscorum* complex (Table 1). Up to now the haploid number is smaller than 15 in only three species: *gredleri, acervorum* and *sphagnicolus*. Furthermore, the parasitic genera associated with the genus *Leptothorax* exhibit an haploid number usually higher than 18: *Doronomyrmex* (including *kutteri* and *goesswaldi;* 18, 23, 25, 26 and 28) and *Harpagoxenus* (18 and 20) (FISCHER 1987). Finally, the other genus of Leptothoraciti (subtribe that will be proposed by FRANCOEUR *et al.*, in prep.) is *Formicoxenus* with $n = 11$, 14 and 15 (FRANCOEUR *et al.* 1985).

In the subgenus *Myrafant* and its parasitic genus group (FISCHER 1987) the haploid number varies mainly between 8 and 14 chromosomes, and secondarily (18 species out of 42; CROZIER 1975, FISCHER 1987, TABER and COCKENDOLPHER 1988) exceed 15 chromosomes. These data seem to confirm the conclusions of other investigations carried out on the biology (BUSCHINGER 1981 and 1987), on the analysis of some enzymes (DOUWES and STILLE 1987) and on comparative morphology (unpublished data) of these two groups of Leptothoracines, as being generically different.

Diploid and triploid males.

Diploid males were recently found for the first time in the genus *Leptothorax*. We observed 28 cases of diploidy among 169 examined males of *L. muscorum* giving the high percentage of 17.6%. Fourteen of these cases are identified on Table 1; the chromosome enumeration for 14 other males was not precise enough to determine a diploid number. All these males were produced by 10 colonies sampled in some regions of Québec and in Utah; eight colonies are pointed out on Table 1 and the two others are CAF 09521 and 10265. In

most of the cases, the diploid number is $2n = 36$; we also found $2n = 44$ and $2n = 30$. FISCHER (1987) described further incidence of diploid males in the parasitic leptothoracines, *Epimyrma stumperi, Doronomyrmex kutteri* and *Harpagoxenus sublaevis*. Even in the latter species two completely triploid males were produced in an inbred strain. Single (up to two per individual) diploid, triploid and tetraploid cells were observed in the testes of many species. In *Chalepoxenus muellerianus* (1 male), *Epimyrma kraussei* (1 male), and *E. ravouxi* (2 males), among haploid cells between 4 and 15 diploid cells were found. It is striking that such completely or partially diploid males occurred in 6 out of 22 parasitic leptothoracine species, but in none of the 43 independent species investigated (FISCHER 1987).

The presence of diploid *L. «muscorum»* males from the very first cycles of the artificial breeding must be underlined here. For example the colony 11183 (Lac-Mégantic, Frontenac co., Québec) has produced three diploid males as soon as the first summer passed in growth chamber; this means that these males were coming from brood produced partly in the field. The method used for the slide preparation (IMAI *et al.* 1977) cannot explain this high frequency of diploid males.

Until now the presence of diploid males still appears as a rare phenomenon in Hymenoptera. Among Formicids, it has been observed in *Pseudolasius* sp. (HUNG *et al.* 1972), *Rhytidoponera* spp. (WARD 1980), *Lasius alienus/niger* (PEARSON 1983) and *Formica pressilabris* (PAMILIO and ROSENGREN 1984). Apparently it is more frequent in *Solenopsis invicta* (ROSS and FLETCHER 1985). Some authors (WARD 1980; PAMILO and ROSENGREN 1984) link the male diploidy with the inbreeding effect. Now, as we have stressed before, the presence of diploid males in *L. muscorum* did not seem to be related with the number of cycles spent in artificial conditions. In the particular case of *S. invicta,* ROSS and FLETCHER (1985; p. 898) wrote that the common occurence of male diploidy in this species is causally related to the founder effect during colonization of North America. At the present time we cannot identify one general cause for this phenomenon.

HUNG *et al.* (1974) specified that the testes of diploid males in *S. invicta* were often atrophied; on the contrary those observed among diploid males of *L. muscorum* had a perfectly normal appearance.

CONCLUSIONS

The chromosome enumeration in different forms presently identified under the species name *muscorum* along with other nearctic species of *Leptothorax* (s.s.) has revealed a complex situation rather difficult to untangle completely from the data now available. Nevertheless they provide a better knowledge of the natural species hidden under the *muscorum* taxon as described by BROWN in 1955.

At leat four species are extracted out of this old name. One of them species A with typically $n = 15$ or 16 is a northern form and its biology shows unusual peculiarities (HEINZE and BUSCHINGER 1987, 1988*a*, 1988*b*, 1989). Species 1 from warmer habitats is presently known only in southwestern U.S. The remaining material of the *muscorum* complex includes a minimum of two species. The karyotype study of FISCHER (1987) suggest a greater number; these results must be confronted to comparative morphology still to be completed. The species *L. crassipilis* and *L. retractus* have, as in some *muscorum* forms, an haploid number of 17 or 18 chromosomes. But these forms are usually not to be confused since they exhibit distinctive morphological features.

It seems that the last glaciations, the precariousness of the exploited microhabitats and possibly other ecological factors like large forest fires had favoured an effervescence of the original genotype of *L. muscorum*. As stated for *Aphaenogaster rudis* by CROZIER (1977) the variations of the genotype are not yet concretized in important variations of the phenotypes. This is evolution indeed.

The variation tendencies observed up-to-now in the chromosome numbers for the species of the traditional subgenera *Leptothorax* and *Myrafant* support the conclusion that these two taxa, when properly emended, represent distinct genera as do together morphological and electrophoresis data.

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